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EFFECT OF *SACCAHAROMYCES CEREVICIAE (INACTIVATED YEAST)* ON QUAIL FED RATION CONTAMINATED WITH FUMONISIN B1 MYCOTOXICOSIS.

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ABSTRACT

In this study, 160 day-old, Japanese quail chicks were randomly assigned into four groups, 40 birds of each. The first control group was fed on a basal diet (group1) and the other 3 groups were fed basal diet supplemented with 0.5% yeast, 0.5% yeast + 200mg fumincin-B1/ kg diet and 200mg fumincin-B1/ kg diet, respectively. The results of fumincin-B1(FBI) supplemented group showed a significant on increase in mortality rate, alanine amonotransferase (ALT), aspartate aminotransferase (AST), cholesterol, uric acid and createnine and a decrease in body weight, hatchability, fertility and hatching weight, hematological parameters, serum total proteins, globulins and glutathione peroxidase (GPx) activity in comparison with the control group. Yeast supplementation was found to alleviate the toxic effects of FB1.

Key Words: *Fumonisin B_1 • Japanese quail, Biochemical, Bloods, Yeast.*

INTRODUCTION

Mycotoxins are secondary toxic metabolites of moulds and their negative effects on poultry production are difficult to overestimate. More than 300 mycotoxins have been shown to induce signs of toxicity in mammalian and avian species. It has been estimated that 25% of the world's crop production is contaminated with mycotoxins (Fink-Gremmels, 1999) and this number is increasing. These contaminants have a broad range of toxic effects, including carcinogenicity, immunotoxicity and neurotoxicity in addition to reproductive and developmental toxicity (Diana *et al.*, 2006; Elaroussi *et al.*, 2006 and 2008). The most important mycotoxins are aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone. (O'Brien and Dietrich, 2005; Butkeraitis *et al.*, 2004, 2006 and Meissonier *et al.*, 2007).

Fumonisin B-1 (FB-1) is a mycotoxin produced by *Fusarium verticillioides*, (Loiseau *et al*., 2007), *Fusarium moniliforme*-contaminated culture material has been shown to induce toxic responses, including increased mortality, reduced size of the bursa of Fabricius, thymus, and spleen, decreased body weight gain, myocardial degeneration, myocardial hemorrhage, alterations in the hemostatic mechanism, and necrosis of hepatocytes, when fed to chickens, ducklings, and turkey poults (Jeschke *et al*., 1987; Ogido *et al*., 2004 and Deshmukh *et al*., 2007).

Recently, yeast has been successfully used as an effective agent for counteracting aflatoxin or reducing aflatoxicosis in the small intestine (Stanley *et al*., 1993) and reducing ochratoxin in male broiler chicks (El-Barkouky, 2008). As well, improve the immune response of poultry (Santin *et al*., 2003). Yildirm and Parlat (2003) showed that, hatchability, fertility and embryonic mortality improved due to adding yeast into laying quail diets.

Therefore the current study aimed to investigate the effectiveness of yeast in countring the harmful effects of fumincin-B1 which was enriched in Japanese quail diets for 9 week of age via performance and physiological parameters.

MATERIALS and METHODS

A total number of 160 one day old, Japanese quail chicks, obtained from the farm of Biological Application Department, Nuclear Research Center at Inshas were used in the present study. The quail chicks were randomly assigned equally to four groups (40 birds per each). Each group contained 2 replicates of 20 birds. For 9 week experimental period, birds were kept in battery cages, water and feed was provided ad lib. A basal diet of 24% protein and 3000 kcal M.E./kg was formulated to cover all nutrients needed a according to N.R.C 1994, (Table 1). The experimental design was included four tested groups as follows:

1- Group(1): The basal diet and served as control

2- Group(2): The basal diet supplemented with 0.5 % yeast.

3- Group(3): The basal diet supplemented with 0.5 % yeast + 200mg fumincin-
B1(dissolved in distilled water) /kg diet.

4- Group(4): The basal diet supplemented with 200mg fumincin B1/kg diet.

Fumonisin production and analysis.

Fusarium moniliforme culture material, containing FB1, was produced as previously described by Weibking *et al.* (1995). Culture were grown in sterilized 0.946 liter canning jars containing 100g of whole shelled corn and 100ml of distilled water. A 5ml vial of lyophilized F.moniliforme M-1325, provided by the Fusarium Research Center (Pennsylvania State University Park,Pa.) was added to 500 ml sterilized distilled water and 2ml of this suspension was added to the autoclaved jars. The jars were incubated in the dark in an open room for a total of 5 wk at 27^0C.

Individual body weight, mortality rate and feed intake were recorded weekly. After sexual maturity at 6 week of old, 12 female and 6 male of each treatment were used. Egg production, fertility, hatchability, embryonic death and weight of hatching chicks were recorded up to 9 weeks of age.

At 6 week old, three birds of each replicate group (6 birds/ group) were randomly slaughtered. Carcass was eviscerated and some organs, including spleen, liver, heart, testes, oviducts and ovaries were removed and weighed individually then their weights were related to live body weight.

Two blood samples were collected during slaughtering the first one was collected with anticoagulant while the second sample was collected without anticoagulant to measure hematological parameters and some serum biochemical parameters. Hematological parameters: Red and White blood cells count (RBC and WBC) were determined according to Natt and Herrick (1952). Hemoglobin concentration (Hb) was determined according to Dacie, and Lewis, (1991*)*. Serum total protein according to Biuret method (Henery, 1964*)*, albumin according to Drupt, (1976). Serum globulin was calculated by subtracting albumins from total protein. Cholesterol was estimated by using the method of Watson, (1960). Liver function tests: alanine amino transaminase (ALT) and aspartate amino transaminase (AST) were determined according to Reitman and Frankel (1957). α-phetoprotein (AFP) was determined by radioimmunoassay (RIA) techniques, and used to estimate GPx activity (nmol/min/ml). Uric acid was determined according to James and White (1971) and Createnine was estimated by using the method of Husden and Ropaport (1968), calorimetrically by using commercial kits (bio Merieux - co., Marcyl Etoile – France).

Statistical analysis:

Data were analyzed statistically using the General Linear Models procedure of the program CoStat (1986). The ANOVA model was used according to Snedecor and Cochran (1982) to study the role of *Sacchromyces Cervisiae* in suppressing the effects of Fumincin mycotoxicosis in Japanese quail. The differences between averages were subjected to Duncan Multiple Range test for all variables (Duncan 1955).

Table 1: Composition and calculated analysis of the basal diets

Contents	Starter diet %
Ground yellow corn	53.00
Soybean meal (44%)	34.00
Corn gluten meal (60%)	7.000
Calcium carbonate	1.400
Dicalcium phosphate	1.600
Sodium chloride	0.400
Vegetable oil	1.500
L-Lysine-Hcl	0.200
DL-Methionine	0.280
Choline chloride	0.240
Premix*	0.380
	100.00
Calculated analysis**	
Crude protein	24.0
ME	3000
Ca	1.01
Available P	0.45
Lysine	1.37
Methionine	0.64
Methionine + Cysteine	1.11

*Each kilogram of diet contains, A, 12000 I.U; D3, 2000 I.U; E, 10mg; K, 2mg; B1, 1mg; B6,1.5mg;B12, 10µg; B2, 4mg; Pantothonic acid, 10mg;Niacin, 20mg; Folic acid, 1g; Biotin, 50µg; and Choline chloride, 500mg.Copper, 10mg; Iodine, 1mg; Iron, 30mg; Manganese, 55mg; Zing, 55mg and Selenium, 1mg.

** Values were calculated according to the nutrient composition to the NRC (1994).

RESULTS

Body weight, and Mortality rate:

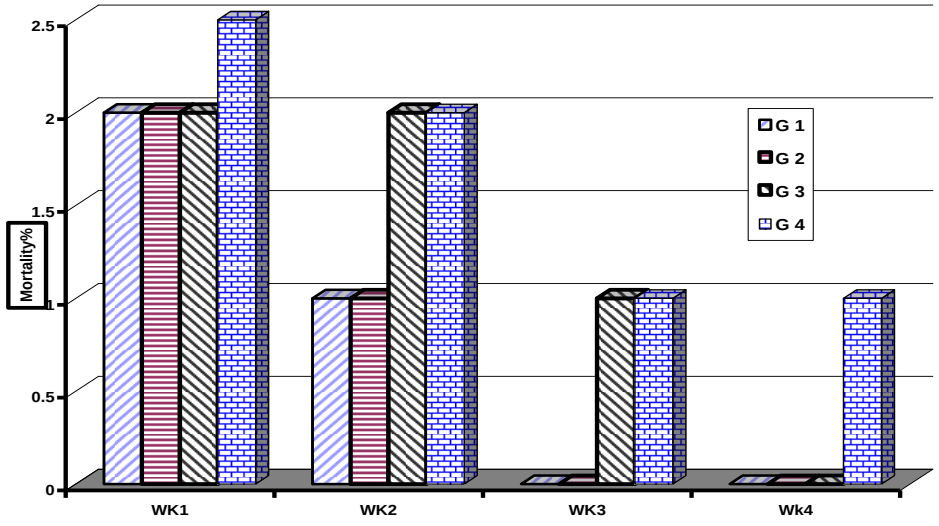
Body weight of birds fed yeast supplemented diet (G2) was significantly higher than that of the control (Table 2) through the experimental periods. The group of birds received fumincin-diet (G4) recorded a significantly lower body weight than all treated groups. Mortality rate of fumincin-diet (G4) and fumincin-yeast group (G3) were higher than other treated groups (Fig 1).

Table 2: Effect of *Sacchromyces Cervisiae* (Yeast) and Fumonisin on Body weight (gm) in Japanese quail

Weeks	G 1 Control	G 2 Yeast	G 3 Yeast +F-B1	G 4 F-B1
WK 1	21.46^{a} ±0.46	22.06^{a} ±1.38	19.48^{b} ±0.49	18.84^{c} ±0.70
WK 2	42.25^{b} ±0.66	47.06^{a} ±1.22	40.68^{c} ±0.68	35.56^{d} ±1.47
WK 3	80.44^{ab} ±1.64	86.35^{a} ±2.38	79.05^{bc} ±1.79	75.80^{c} ±1.09
WK 4	131.71^{b} ±1.24	136.10^{a} ±2.13	124.73^{c} ±0.46	122.89^{d} ±1.19
WK 5	174.38^{b} ±3.90	180.49^{a} ±1.81	161.43^{c} ±3.08	149.90^{d} ±4.28
WK 6	197.96^{ab} ±1.58	209.01^{a} ±3.37	190.59^{bc} ±0.94	182.28^{c} ±1.51
WK 7	243.60^{b} ±8.10	263.49^{a} ±12.21	221.26^{c} ±7.40	201.68^{d} ±8.88

$^{a, b, c}$ Means within row with different superscripts are significantly different ($p < 0.05$)

Fig.(1) The effects of Yeast and Fumonisin on Mortality in Japanese quail



Hatchability, Fertility and Hatching weight:

It is clear that, addition of yeast into basal diet improved egg fertility and hatchability, in contrast enriched FB1 (G4) led to higher depression in both parameters, (Table 3).

Table 3: Effect of Yeast and Fumonisin on Hatchability%, Fertility %, Infertility % and Hatching weight (gm) in Japanese quail

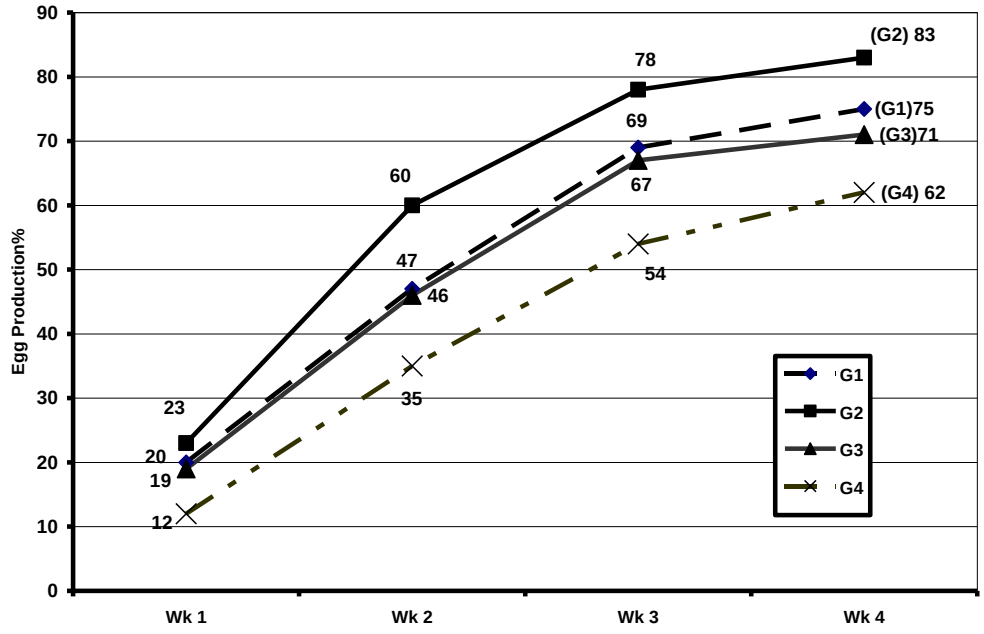
Weeks	Item	G 1 Control	G 2 Yeast	G 3 Yeast +F-B1	G 4 F-B1
WK 1	Hatchability%	75	76.64	70.37	40
	Fertility %	79.41	93.10	78.50	66.70
	Hatching weight(gm)	6.80^{a} ±0.10	6.81^{a} ±0.21	6.21^{b} ±0.09	6.19^{b} ±0.10
WK 2	Hatchability%	77	79.3	76.92	58.33
	Fertility %	89.28	83.94	88.88	73.33
	Hatching weight(gm)	8.66^{a} ±0.11	8.62^{a} ±0.09	8.13^{b} ±0.14	7.87^{c} ±0.11
WK 3	Hatchability%	79.30	80.60	77.20	61.76
	Fertility %	90.3	96	89	72.8
	Hatching weight(gm)	8.83^{a} ±0.14	8.87^{a} ±0.13	8.32^{b} ±0.10	7.89^{c} ±0.12

$^{a, b, c}$ Means within row with different superscripts are significantly different ($p < 0.05$)

Egg production percentage:

Egg production percentage of laying quails fed yeast (G2) was significantly higher than the control group (Figure2). Mean egg production % were lower in birds fed Fumonisin (FB1) group 4.

Fig.(2): The effects of Yeast and Fumonisin B1 on Egg Production in Japanese Quail.



Carcass aspects and internal organs.

There are significant increases in carcass heart, spleen, testis and ovary in group 2 and significant decrease in group 4 (Table 4) compared to the control either in males or females. Relative weight of liver increases ($p < 0.05$) in the group G4 with fumincinB1 in comparison with the control.

Table 4: Effect of *Sacchromyces Cervisiae* (Yeast) and Fumonisin on Carcass weight and relative weights of some organs in Japanese quail

Groups	Carcass		Liver		Heart		Spleen		Testis	ovary	Oviduct
	♀	♂	♀	♂	♀	♂	♀	♂			
G 1 control	64.68^{b} ±1.16	74.06^{a} ±0.78	3.86^{c} ±0.12	2.34^{c} ±0.19	1.35^{a} ±0.45	0.75 ±0.10	0.080^{b} ±0.007	0.090^{a} ±0.007	2.47^{a} ±0.28	2.21^{b} ±0.26	2.42^{b} ±0.06
G2 Yeast	68.38^{a} ±1.27	75.71^{a} ±0.07	3.11^{d} ±0.09	2.45^{c} ±0.04	1.24^{a} ±0.33	0.92 ±0.04	0.102^{a} ±0.01	0.093^{a} ±0.005	2.49^{a} ±0.14	2.61^{a} ±0.06	2.34^{b} ±0.20
G 3 Yeast+FB1	64.24^{b} ±0.83	73.39^{b} ±1.05	3.99^{b} ±0.11	2.70^{b} ±0.27	0.76^{b} ±0.11	0.71 ±0.09	0.080^{b} ±0.004	0.074^{b} ±0.005	2.39^{b} ±0.02	1.72^{c} ±0.13	2.79^{a} ±0.04
G 4 FB1	61.65^{c} ±0.46	72.99^{c} ±0.15	4.16^{a} ±0.19	3.02^{a} ±0.01	0.64^{b} ±0.03	0.89 ±7.07	0.074^{b} ±0.004	0.060^{c} ±0.006	1.95^{c} ±0.02	1.01^{d} ±0.18	0.81^{c} ±0.09

$^{a, b, c}$ Means within row with different superscripts are significantly different ($p < 0.05$)

Some Blood parameters:-

Hematological and Proteins Parameters of the different groups are presented in Table (5). The Red blood cells count, white blood cells count (WBCs) and Hemoglobin value are increased significantly due to adding yeast into diets than control. Also mean values of hemoglobin, white blood cells count, and total erythrocyte count were slightly higher in birds fed FB1. At the same table, the results obtained for total proteins, albumin, globulins and A / G ratio (Table 5) indicated that adding yeast (G2) to the basal diet resulted in a significant increase in total proteins and globulins. G3 come next to G2 in increasing total proteins and globulins, while the control group (G1) showed the significant decrease slightly values. While G4 was increased slightly in quails fed FB 1 than control. Also tables (5) shows significant increase in ALT, AST, cholesterol, uric acid, createnine and alpha feto protein (AFP) and decrease in GPx activity in quails received fumonisin (G4) in comparison with the control group one (G1) and all treatments.

Table 5: Effect of *Sacchromyces Cervisiae* (Yeast) and Fumonisin B1 on Some Biochemical Parameters in Japanese quail

Groups	RBCs (-xmm^{6})	WBCs (-xmm^{3})	Hb (g/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT (u/I)	AST (u/I)	uric acid (mg/dl)	Createnine (mg/dl)	Cholesterol (g/dl)	GPx activity (nmol/ min/m)l
G 1 control	3.49^{b} ±0.20	16.1^{c} ±0.74	11.14^{b} ±0.40	4.06^{b} ±0.21	1.75 ±0.12	2.31^{ab} ±0.19	10.24^{bc} ±1.32	25.38^{c} ±0.87	3.72^{bc} ±0.08	0.47^{c} ±0.12	125.4^{b} ±1.67	74.20^{b} ±9.41
G2 Yeast	4.12^{a} ±0.39	20.24^{a} ±0.67	13.2^{a} ±1.39	4.78^{a} ±0.19	1.69 ±0.15	2.99^{a} ±0.28	9.42^{c} ±0.58	24.20^{c} ±0.83	3.50^{c} ±0.22	0.46^{c} ±0.11	123.6^{bc} ±2.23	98.00^{a} ±9.56
G 3 FB1 +Yeast	3.94^{ab} ±0.28	17.2^{b} ±0.72	11.98^{ab} ±0.74	4.68^{a} ±0.15	1.78 ±0.13	3.00^{a} ±0.26	11.22^{b} ±0.67	27.20^{a} ±2.16	3.80^{b} ±0.25	0.79^{b} ±0.15	127.6^{a} ±1.14	59.38^{c} ±5.82
G 4 FB1	3.50^{b} ±0.22	16.52^{bc} ±0.39	11.70^{ab} ±0.99	4.18^{ab} ±0.14	1.44 ±0.37	2.74^{ab} ±0.26	12.58^{a} ±0.91	30.60^{a} ±2.70	4.43^{a} ±0.22	1.07^{a} ±0.17	129.6^{a} ±2.07	34.20^{d} ±3.89

$^{a, b, c}$ Means within row with different superscripts are significantly different ($p < 0.05$)

DISCUSSION

Body weight, and Mortality rate:

In the present study showed that body weight of birds fed yeast supplemented diet was significantly higher than that of the control through the experimental periods. The group of birds received fumincin-diet recorded a significantly lower body weight than all treated groups. Mortality rate of fumincin-diet and fumincin-yeast group were higher than other treated groups.

These results were expected due to role of mycotoxin FB1 (higher than 100mg/kg diet) in depressing body weight gain of chicks (Brown *et al*., 1992; and Henry *et al*., 2000). Furthermore, Asroni *et al*. (2006); Elbarkouky, (2008); Girish and Smith, (2008) and Sharma *et al*. (2008) observed a reduction in body weight gain of Japanese quails fed fumincin-B1 diet.

Javed *et al*. (1993) observed an increase in mortality rate of broilers fed FB1-supplemented diets. The beneficial effect of yeast in countering the harmful action of fumincin-B1 has been reported by Abdel-Azeem *et al.* (2005). In addition Stanley *et al*. (1993) and Abu-Taleb *et al*. (2007a) related the beneficial role of yeast due to its structure of cell wall which adsorbed mycotoxin preventing its adverse effect.

Hatchability, Fertility and Hatching weight:

It is clear that, addition of yeast into basal diet improved egg fertility and hatchability, in contrast enriched FB1 led to higher depression in both parameters. The presence of yeast inherent Fumonisin enriched diet success to improve fertility, hatchability of quails than those fed FB1 alone. Yildirm and Parlat (2003) showed that, hatchability, fertility and embryonic mortality are improved due to adding yeast into Japanese quail diets.

Egg production percentage:

Egg production percentage of laying quails fed yeast was significantly higher than the control group. Mean egg production % were lower in birds fed Fumonisin (FB1) group. The improvement in laying performance may be due to the decreasing effect of yeast on pathogenic bacteria, Kim *et al*. (2002) and Park *et al*. (2002). Soliman (2003) stated that, including live yeast to laying diets improved egg production and laying performance.

Carcass aspects and internal organs.

The quail chicks fed diets with yeast showed significant increases in carcass and relative wights of heart, spleen, testis and ovary and significant decrease in fed diet contaminated with fumincin B1when compared with the control either in males or females. Relative weight of liver increases ($p < 0.05$) in the group G4 with fumincinB1 in comparison with the control. *Fusarium moniliforme*-contaminated culture material has been shown to induce toxic action, including increased mortality, reduced size of the bursa of Fabricius, thymus, and spleen, (Ogido *et al*., 2004 and Deshmukh *et al*., 2007). On the other hand, live yeast, *Sacchromyces cerevisiae*, was found to alleviate the adverse effects of mycotoxins in poultry (Abu Taleb *et al*., 2007b).

Some Blood parameters:-

The Red blood cells count, white blood cells count (WBCs) and Hemoglobin value are increased significantly due to adding yeast into diets than control. Similar results were recorded by Wakwak *et al*. (2003) who observed an increase in WBCs of growing quails fed diets with live yeast. The increment in indicated that, adding yeast into laying quail diets may improve immunity system. Santin *et al*. (2003) reported that the immune response of birds improved by adding yeast into diets. G4 was increase slightly in quails fed FB 1 than control. Mean values of hemoglobin, white blood cells count, and total erythrocyte count were slightly higher in birds fed FB1. Total leukocyte counts were higher in FB1-fed group because of an increase in the number of heterophils and lymphocytes, Deshmukh *et al.* (2007) and Sharma *et al.* (2008).

The above mentioned results in this study showed that a significant increase in total proteins and globulins in yeast treated group (G2). While G3 come next to G2 in increasing total proteins and globulins, but the control group (G1) showed the significant decrease slightly values. While G4 was increased slightly in quails fed FB 1 than control. Some authors reported that Japanese quail (Abdel-Azeem *et al.*, 2005), and broiler chicks (Tollba *et al.*, 2004), showed significant increases in the values of total proteins and globulins as the result of feeding Yeast. Sharma *et al*. (2008). Showed that total serum proteins, albumin and globulins values were higher in all treatment groups of birds fed FB1 compared with the control group.

The significant increases in ALT, AST, cholesterol, uric acid, createnine and alpha feto protein (AFP) and decrease in GPx activity in quails received fumonisin (G4) in comparison with the control group one (G1) and all treatments. Yeast observed partial improves the parameters in group three (G3). The previous results agree with those of Sharma, *et.al*.,**(**2008) they reported that birds fed FB_1 showed higher values of cholesterol, aspartate transaminase, lactate dehydrogenase, and creatine kinase than that of the control group.

Glutathione is probably the most abundant natural low molecular weight thiol that is detected in virtually all living cells. It plays a central role in transporting amino acids across cell membranes, catalyzing disulfide exchange reactions, and serving as coenzymes for certain enzymes, detoxifying free radicals and acting as reactive intermediates (Sen and Packer, 1996 and Mohamed, 2000). The liver appears to be a vast reservoir for GSH, it releases from the hepatocytes into bile and blood where it turns over rapidly to be available to other organs (Kaplowitz *et al*., 1994).

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تأثير اضافة ساكاروميسيس سيرفيسى (خميره غير النشطه) على السمان المغذى على علائق ملوثه بسموم الفيومينسين ب1

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استهدف إجراء هذا البحثُ دراسة تأثيرِ إضافة الخميرة لتثبيط سموم الفيومينسين ب 1 لعلائق السمان الياباني على النمو وعلى مواصفات الذبيحة وبعض مكونات الدم. تمَّ استخدامُ 160 طائر من السمانِ الياباني عمر يوم وقسمتْ عشوائياً إلى اربع مجاميع في كل مجموعة 40 طائر . المجموعة الأولى : المجموعة الضابطة تم تغذيتها على العليقة الاساسية بدون اضافات (العليقة العادية). المجموعة الثانية : تم تغذيتها على العليقة الآساسية مع اضافة 0.5% خميرة. المجموعة الثالثة : تم تغذيتها على العليقة الآساسية مع اضافة 0.5 % خميرة + 200 مجم فيومينسين ب 1 لكل كيلوجرام عليقة المجموعة الرابعة : تم تغذيتها على العليقة الاساسية مع اضافة 200 مجم فيومينسين ب1 لكل كيلوجرام عليقة. تم تسجيل وزن الجسم والنفوق أسبوعيا ولمدة سبع أسابيع ، تسجيل انتاج البيض ونسبة الفقس والخصوبة ووزن السمان الفاقس من الاسبوع السادس وحتى الاسبوع التاسع (علما بأن انتاج البيض عند 42 يوم ومدة الفقس 17 – 19 يوم). في الأسبوع السادس تمَّ ذبح ستة طيور سمان من كل مجموعة وتمَّ جمع الدمْ لعد كرات الدم الحمراء و البيضاء وتقدير الهيموجلوبين وكرات الدم المضغوطة وكذلك تقدير بروتين الدم والالبيومين والجلوبيولين والجلوتاثيون وبعض وظائف الكبد والكلى وكذلك الكوليستيرول. كما تم وزن بعض الأعضاء الدَّاخلية للسِّمان . أوضحت النتائج المُتَحصل عليها أنَّ إضافة الخميرة في هذه الدراسة كان مؤثراً وأعطى زيادة معنوية في وزن الجسم وانتاج البيض وتحسين فى نسبة الفقس والخصوبة ووزن السمان الفاقس. وكذلك عد كراتِ الدم الحمراء والبيضاء والهيموجلوبين وكرات الدم المضغوطة والبروتين الكلى والجلوبيولينات وانخفاض فى النفوق نتيجة تثبيط التأثيرات السامة للفيومينسين ب1.